SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. hiN cells derived from fibroblast cultures express a panel of neuronal markers. Related to Figure 1.

(A-G) Additional examples of hiN cell morphology and immunostaining, as in Figure 1. hiN cells stain positively for the neuronal markers Tuj1, MAP2, Tau1, NeurN, vGLUT1, Neurofilament-160 kd, and NCAM, whereas staining for the astroglial marker GFAP is not apparent. The fibroblast lines used for hiN cell reprogramming herein are STC0022 (A-A'', C-C'', E-E'', G-G'' and I-J) and AG00768 (B-B'', D-D'',F-F'', H-H'', K, L, and M). Antibodies used are MAP2 (A', B', E and F; in green), Tuj1 (A-D, G, H, and M; in red), Tau1 (C' and D'; in green), NeuN (E' and F'; in red), vGLUT1 (G' and H'; in green), Neurofilament-160 kd (NF; I and K; in green), neuronal cell adhesion molecule (NCAM; J and L; in green), and glial fibrillary adhesion molecule (GFAP; M; in green). hiN cell cultures were analyzed at 3 weeks (A-H) or 6 weeks (I-L) after transduction. In (M), nuclei are counterstained with DAPI; M, inset shows GFAP-positive positive control staining in a rat astrocyte culture. Merged images and scale bars are labeled as indicated.

Figure S2. Further analysis of hiN cell conversion. Related to Figure 2.

(A) RT-PCR analysis of exogenously transduced viral conversion factors. Expression of virally transduced Ascl1, Brn2, and Myt1l in fibroblasts (STC0022) at 0 and 3 weeks after hiN induction with the 5-factor viral cocktail.

(B) Temporal profile of total neurite process length in 5-factor hiN cell cultures. Total neurite length per cell was determined using Image J software tools. n= 3-4 at each time point.

(C) Temporal profile of cell survival in the context of hiN cell 5-factor transduction or transduction with empty vector. Total numbers of cells per well at the indicated time point after

5-factor transduction, as determined by Hoechst nuclear staining, are presented. N = 3 per group.

(D) Required extrinsic soluble factors in hiN cell conversion. Fibroblasts were transduced with the 5-factor cocktail for hiN induction, and the indicated soluble factor was left out from the hiN cell conversion protocol as in Figure 1A. Time points of cell maintenance post-transduction are as indicated; data are presented as a percent of the complete 5-factor protocol ('All') at 21 days post-transduction. Cells were stained for expression of vGLUT1. Only BDNF appears essential. N = 3 per group.

(E-F) Cleaved caspase-3 staining of hiN cells induced with all factors (E) or lack of BDNF (F). Cultures as in (D) were examined for evidence of apoptosis by staining for cleaved caspase-3 (c-caspase 3) nuclear expression. Cells at day 21 post-transduction were co-stained with an antibody for c-caspase 3 (in red) and with the general nuclear marker Hoechst 33258 (light blue). Scale bar, 30 μm.

(G) Quantification of cleaved caspase-3-positive cells as above. n=3 per group. $*$, $P < 0.05$.

(H-J) Flow cytometric analysis of hiN cell cultures at 3 weeks after 5-factor transduction (I, J) or untransduced fibroblasts (H) stained with an antibody specific for human NCAM (J) or without a primary antibody (I). A population of NCAM-positive cells is apparent only in the stained hiN cell cultures (in pink).

(K) Schematic representation of the polycistronic pHAGE2-EF1α-ABZ lentiviral vector. The engineered hiN gene cassette consists of a single polycistronic RNA encoding the 3 cDNAs as indicated, transcribed under the control of the EF1α promoter. See extended experimental procedures for details.

(L-Q) Immunohistochemical analysis of hiN cell cultures generated with the pHAGE2-EF1α-ABZ lentiviral vector. Double immunocytochemistry of hiN cells with antibodies to MAP2 (red) and Ascl1 (green) 14 days after hiN cell induction with the polycistronic vector only (L-N), or this

vector along with a Myt1l lentiviral vector (O-Q). The majority of Ascl1-positive cells expressed MAP2. Arrowhead indicates MAP2/Ascl1 double-positive cells. Nuclear staining with Hoechst 33258 is shown in blue. Scale bar, 50 μm.

(R-V) Immunocytochemistry of polycistronic vector-generated hiN cells with antibodies against MAP2 (R), Tau1 (S), Tuj1 (T), Tbr1 (U) and vGLUT1 (V) 14 days after hiN cell induction with ABZ-polycistronic and Myt1l lentiviral vectors. Scale bars, 50 μm (R) 20 μm (T) and (V).

Figure S3. Hierarchical clustering analysis of hiN cell and human neuron transcriptome gene expression profiles. Related to Figure 2.

Top: Hierarchical clustering was performed to broadly compare the transcriptome profiles generated from the purified hiN cells (as in Figure 2D) with a large set of 336 existing profiles of human neurons and other cell types that are publically available on the NIH Gene Expression Omnibus Dataset repository (GEO; http://www.ncbi.nlm.nih.gov/geo/). Zooming in to the text at the top of the figure allows visualization of column identifiers for the individual samples at the top of the figure, which are further described in Table S4. The publically accessible gene expression array profiles had been generated from cells using a variety of methods, including laser micro-dissected neurons from human brain, and in the context of a variety of independent studies (See Table S4). Hierarchical clustering was conducted using Pearson's correlation coefficient and complete linkage based on differentially expressed transcripts using a stringent binary absent/present analysis (see Extended Experimental Procedures). To simplify visualization of this large data set, categories of cell populations are color coded just below the sample identifiers: GEO datasets of human neurons are indicated by red bar, neuronal progenitors by pink bar, astrocytes in gray bar, fibroblasts in dark blue bar, and pluripotent stem cells by green bar. Datasets from the present study (as per Figure 2D) are indicated for fibroblasts (light blue bar; at far left), mixed hiN cell cultures (unsorted, yellow bar; at far right) and FACS-sorted hiN cells (orange bar at far right, between human neurons [red bar] and the

mixed hiN cell cultures [yellow bar]). A dendrogram is presented below the datasets, based on the hierarchical clustering algorithm. The dendrogram subtree (branches) containing the hiN cell samples and all human neuron samples is highlighted in pink (whereas remaining subtree branches are in blue). This clearly demonstrates that the FACS-sorted hiN cell samples cluster most closely with the neuronal cell samples, compared with all other sets.

Bottom: A heat map is presented. The 710 gene transcript probe sets that are included in the analysis are those absolutely differentially expressed in the Figure 2 samples: that is, either present in all of the purified hiN cell samples in figure 2 (but not in any of the fibroblast samples), or present in all fibroblast samples (but not any of the sorted hiN cell samples). Transcript probe sets are identified by row using Affymetrix probe set annotation, as listed on right. In the heat map, red squares indicates probe sets that are present in a given sample column, whereas black squares indicate absent probe sets in the sample set for each column. For visualization purposes, columns corresponding to the highlighted pink dendrogram subtree (that includes the hiN cell samples and neurons) are shaded darkly, whereas all other sample columns within the heat map are shaded lightly.

Figure S4. Identification of transplanted hiN cells in vivo. Related to Figure 5.

(A) GFP-labelled hiN cells were visualized by fluorescent microscopy within acutely prepared brain slices of P7 to P20 mice after *in utero* transplantation. A red fluorescent dye, Alexa-598, was included in the patch recording pipette buffer for facile identification of recorded cells. Fluorescence images are shown using an excitation wavelength of 470 nm (for GFP; see corresponding cell recording in Figures 5G to 5J). Arrow points to recording pipette.

(B) The red fluorescent dye Alexa-594 was present within the holding pipette solution to allow visual identification of the patched cell. 5 min after whole cell access, the cell in (A) was re-imaged for red fluorescence (using an excitation wavelength of 590 nM). Arrow points to recording pipette.

(C) Subsequent to electrophysiological recording from the acutely prepared brain slice (as in Figures 5G to 5J), the slice was processed by paraformaldehyde fixation followed by immunostaining with an antibody for the human-specific mitochondrial antigen (hmito). Multicolor confocal fluorescence imaging confirmed that the GFP^+, $Alexa\text{-}598^+$ neuron in (A) and (B) stained positively with the hmito antibody (n=3).

Figure S5. Further characterization of APP processing in hiN cell cultures. Related to Figure 6.

(A) Time course gene expression profiles for synaptophysin in UND and FAD hiN cell cultures. Samples were collected at 0, 1, and 3 weeks after gene transduction with the 5 hiN cell conversion factors, as indicated. All analyses were by quantitative real-time RT-PCR. Expression was comparable in the UND and FAD cultures. All data were normalized to GAPDH expression. $*$, $P < 0.05$; n=9.

(B) Quantification of average cell number per well in hiN cell cultures at 3 weeks, using Hoechst nuclear staining. Individual lines are as listed in Supplementary Table 3.

(C, D) Time course gene expression profiles for APP (C) and BACE1 (D) in UND and FAD hiN cultures. Samples were collected at 0, 1, and 3 weeks after gene transduction with the virally encoded conversion factors, as indicated. All analyses were by quantitative real-time RT-PCR (see Extended Experimental Procedures). Expression was comparable in the UND and FAD cultures. All data were normalized to GAPDH expression. $*$, $P < 0.05$; n=9.

(E-G) Confirmation of specificity of antibodies to Aβ40 or Aβ42 by dot blot analysis using synthesis human Aβ40 and Aβ42 peptides. Peptides (10 pg/10 µl) were spotted onto nitrocellulose membrane and incubated with the antibody as indicated (E; outlined by yellow dashed circle). Standard concentration curves are plotted for Aβ40 (F) and Aβ42 (G) sandwich ELISA assays.

(H-J) Immunocytochemistry (ICC) staining of hiN cell cultures with an antibody to Aβ40 (see Figure 6D). hiN cell cultures were costained with antibodies to Aβ40 and MAP2 (as in Figure 6D except using Aβ40 instead of Aβ42 antibody). Aβ42 (J; from Figure 6D) and Aβ40 (I, from Figure S5H) immunostaining fluorescence was quantified within MAP2-positive ('neuron-like') and MAP2-negative ('fibroblastic') cells in terms of total Aβ40 (I) or Aβ42 (J) pixel intensity per cell using Image J software (NIH). Immunostaining fluorescence pixel intensities were quantified for each of the 6 hiN cell cultures (3 FAD and 3UND, as per Table 1); data presented are aggregated into FAD and UND groups. Results represent as the mean ± SEM (n=35-50 cells per well per MAP2-positive or MAP2-negative group, with 3-4 independent wells per line). $*$, $P < 0.05$.

Figure S6. Altered APP-positive endocytic morphology in FAD hiN cells. Related to Figure 7.

(A-B) Determination of APP-positive puncta diameter (A) and number (B) in each UND and FAD hiN cell culture as labeled. Puncta diameter was quantified by Image J software; see Extended Experimental Procedures for details. Results represent mean ± SEM (n=12-38 cells in a total of 6 wells per group). Data in Figure 6C are derived by the formula (Total puncta area/cell) = (number of puncta/cell) X π(mean puncta diameter/2)^2.

(C-H) Subpopulations of APP-positive puncta (in red) are co-stained with the late endosome marker mannose-6-phosphate receptor (MPR) or the lysosomal marker LAMP2 (in green) in UND (C, F) and FAD (D, G) cultures. Colocalization is visualized as yellow in the merged images. Inset panels present merged as well as individual staining patterns, for visualization of areas as indicated by a blue square. In panel D, an hiN cell with typical neuronal morphology (marked 'n') is present adjacent to a cell with a fibroblastic morphology (marked 'f'). Quantification of data for APP and MPR colocalization area for each of 6 hiN cell cultures from

UND or FAD individuals is presented in (E); quantification of APP and LAMP2 colocalization area for each of 6 hiN cell cultures from UND or FAD individuals is presented in (H). Puncta are defined here as distinct signal intensities less than 1 μm diameter using image J software. Results represent the means ± SEM (n=35-48 cells in 3-6 independent wells per group).

(I) To complement the studies presented in Figures 7J to 7L (which used the Covance BACE1 antibody), FAD cultures were co-stained with a second BACE1-specific antibody (3D5; a gift from Dr. Robert Vassar) together with APP as per Figure 7K. Colocalization is visualized as yellow in the merged images. Inset panels present merged as well as individual staining patterns, for visualization of areas as indicated by a blue square. Staining with this second BACE1 antibody is consistent with the data using the Covance antibody.

(J) Quantification of total APP in FAD and UND hiN cell cultures by immunocytochemistry (using the N-terminus specific antibody 22C11). APP immunostaining fluorescence was calculated by APP pixel intensity per cell using Image J software (NIH). Immunostaining fluorescence pixel intensities were quantified for each of the 6 hiN cell cultures (3 FAD and 3UND, as per Table 1). Results represent as the mean ± SEM (n=60-80 cells per well, with 3-4 independent wells per line). Consistent with the RT-PCR and ELISA data, APP does not appear to be upregulated in FAD versus UND hiN cell cultures. This contrasts with ICC analysis of Aβ40 (Figures S5H and S5I) and Aβ42 (Figures 6D and S5J) in the hiN FAD and UND cell cultures.

(K, L) Quantification of Aβ in media from cultures treated with the γ-secretase inhibitor DAPT (10 μM). Results represent the means ± SEM (n=3-6 per group). $*$, $P < 0.05$.

(M-P) Determination of APP-positive puncta diameter and number in each UND and FAD hiN cell culture, as labeled. Separate quantification of APP-positive puncta diameter (M, O) and number per cell (N, P) under the γ-secretase inhibitor DAPT (10 μM) treatment or wild type PSEN1 over-expression condition (these data were then used to calculate Area as in figure 7O). Results represent the means ± SEM (n=35-50 cells in 3 independent wells). $*$, $P < 0.05$.

SUPPLEMENTARY TABLES

Table S1. Summary of individual hiN cell cultures and corresponding skin fibroblast of origin. Related to Figure 1.

All skin fibroblast lines were derived from de-identified, banked tissue samples; there was no interaction with subjects, no intervention, and private, identifiable information was not collected. STC0022 and STC0033 were obtained from the Columbia University Taub Institute New York Brain Bank. Other cultures were obtained from Coriell Institute (Camden, NJ, USA) and details are available at http://ccr.coriell.org/. Diagnosis is based on clinical diagnosis from Coriell or the New York Brain Bank.

	Line	Patients
UND	STC0022	65yo F
	AG07871	49yo F
	AG07926	N/A[a] F
FAD	AG09908	81yo F PSEN2 (N141I)
	AG06840	56yo M PSEN1(A246E)
	AG07768	31yo F PSEN1(A246E)
SAD	AG06264	62yo F
	AG06263	67yo F
	STC0033	81yo M

[a]Culture was derived from spouse of an AD patient, precise age data unavailable.

Table S2. Differentially expressed transcripts in hiN cells relative to fibroblasts (see attached Spreadsheet). Related to Figure 2.

Listing of Affymetrix probesets (in rows) that were differentially expressed in FACS sorted hiN cell RNA preparations versus fibroblast preparations (false discovery rate (FDR) <25% using B-statistics of the affylmGUI R package). The first 2 columns present the Affymetrix probe set identifier and the Gene Symbol annotation associated, if any, respectively. The log-scaled expression value for each Affymetrix probeset (in rows) is then presented for each RNA sample (in columns titled as per Figure 2D). Also presented are the log-scale fold change (M) in expression values for FACS sorted hiN cells versus fibroblasts; the overall average level (A) in fibroblasts and FACS-sorted hiN cell samples; the False Discovery Rate after multiple hypothesis correction (FDR); and the B-statistic value (B; the log of odds that the gene is differentially expressed, adjusted for multiple comparisons).

Table S3. Incorporation of hiN cells after in utero transplantation. Related to Figure 5.

Cells were transplanted at E13.5, and brains were analyzed at the indicated postnatal day. The brains were serially sectioned, and cells incorporated into brain parenchyma were counted (located at least 50 μm from the ventricular wall). Indicated are the maximum number of cells on a 50-μm section from at least three sections per brain region. -, no cells; +,1-10 cells ; + +,11-50 cells. OB, olfactory bulb; CTX, cortex; SPT, septum; HC, hippocampus; TH, thalamus; HT, hypothalamus; MB, midbrain; CB, cerebellum; BS, brain stem.

Animal	Age	OB	CTX	SPT	HC	TH	HT	MB	CB/BS
413.1	P0	-	+ +	+ +	+	+	-	-	-
413.2	P0	-	-	+	+	-	-	-	-
413.3	P0	-	-	+ +	-	+	+	-	-
413.4	P0	-	-	+	+	+	-	+	-
414.1	P9	-	-	+	-	-	-	-	-
414.2	P12	-	+	-	-	-	-	-	-

Table S4. Description of source transcriptome gene expression Gene Expression Omnibus profiles used for clustering analysis (see attached spreadsheet). Related to Supplementary Figure S3. Right column lists the 336 sample labels of GEO datasets (as per the columns in Figure S3). Second column specifies the cell type as per the GEO database. The third column specifies the GEO Accession Number, which can be used to access additional details for each sample (http://www.ncbi.nlm.nih.gov/geo/).

EXTENDED EXPERIMENTAL PROCEDURES

Plasmid construction and Lentiviral production

cDNA of the five reprogramming factors used here -- Ascl1, Brn2, Myt1l, Oligo2 and Zic1 -- were obtained from Addgene. Inserts were PCR cloned into the lentiviral vector construct pLenti6.3/V5-Dest (Invitrogen) by LR clonase reaction using standard Gateway Technology cloning techniques (Macleod et al., 2006).

To generate a polycistronic vector for expression of Ascl1, Brn2, and Zic1, we used a modified pHAGE2-EF1α vector (Sommer et al., 2009). A DNA fragments consisting of cDNAs for murine Ascl1 and Brn2, separated by an intervening sequence encoding the F2A peptide, was generated by overlapping polymerase chain reactions using AccuPrime™ Taq DNA Polymerase (Invitrogen) as per the manufacturer's instructions. PCRs were carried out using the primer pairs Ascl1 5' XbaI/Ascl1-F2A 3' and F2A-Brn2 5'/Brn2 3' Sal1 (see below), and with the lentiviral single gene vectors above as substrate. Aliquots of the two purified amplicons were then mixed in a 1:1 ratio and used in a second PCR round with the primers Ascl1 5' XbaI and Brn2 3' Sal1. The resulting fragment (Ascl1-F2A-Brn2) was gel-purified and inserted by directional cloning into Xba1 and Sal1-digested pHAGE2-EF1α-Oct4F2AKlf4-IRES-Sox2E2AcMyc upstream of an internal ribosome entry site (IRES) element. Next, a Zic1 cDNA fragment was obtained by PCR with primers pairs Zic1 5' NdeI and Zic1 3' ClaI. This fragment was then inserted between the NdeI and ClaI sites, downstream of the IRES element, of the pHAGE2-Ascl1F2ABrn2 vector. The final construct structure was confirmed by sequencing. Ascl1 5' XbaI, CACCGTCTAGAACCATGGAGAGCTCTGGCAAGATGGAGAGTG; Ascl1-F2A 3', CTTGAGAAGGTCAAAATTCAAAGTCTGTTTCACGCCACTTCCGT TGAACCAGTTGGTAAAGTCCAGCAGCTC; F2A-Brn2 5', AAACAGACTTTGAATTTTGACCTTCTCAAGTTGGCGGGAGACGTGGAGTCCAACCCAGGG CCCATGGCGACCGCAGCGTCTAACCACTA; Brn2 3' Sal1,

TTTGTCGACTCACTGGACGGGCGTCTGCACC; Zic1 5' NdeI;
TGCCATATGATGCTCCTGGACGCCGGA; Zic1 3' ClaI,
GGTTTATCGATTTAAACGTACCATTCGTTAAAATTGGAAGAGAGCGCGC
TGT.

hiN cell induction and transfection

Fibroblasts were plated at 20,000 cells/well in 24-well plates one day before infection and maintained in standard fibroblast media (Dulbecco's minimal essential medium [DMEM] with 10% fetal bovine serum). Culture plates and dishes were treated with Poly-L-Ornithine (Sigma) and Laminin (Invitrogen) before the application of the cells as per the manufacturer's instructions. Fibroblasts were transduced with replication-incompetent, VSVg-coated lentiviral particles encoding Ascl1, Brn2, Myt1l, Oligo2 and Zic1, in fibroblast media containing polybrene (8 µg/ml). Each lentiviral type was added at a multiplicity of infection ~2:1. Two day after transduction, the media was replaced with glial-conditioned N2 media (GCM; N2 media is DMEM/F12 with N2 supplement; Invitrogen) containing 20 ng/ml BDNF and 20 ng/ml NT3 (Peprotech). For glial conditioned media, primary cultures of type 1 astroglia were prepared from the cortices of P1 rat pups using standard techniques (Kaech and Banker, 2006), and these were subsequently cultured in N2 media for 4 days (Kaech and Banker, 2006); media was harvested and filtered through a 45-micron filter (Corning) and used immediately. For the first 4 days in N2 media, dorsomorphin (1 µM; Stemgent) was applied to the culture. Media was changed every 2-3 days for the duration of the culture period. Cells were transfected with pLenti6.3/V5-Presenilin1 and pEGFP-C1 plasmids (9:1) using the Lipofectamine^{TM} 2000 reagent (DNA:LF2000 1 µg : 5 µl in each well of 24 well, Invitrogen). In presenilin wild type rescue experiment to FAD hiN cells, plasmids-transfected cells were incubated for an additional 48-72 h before fixation.

Immunocytochemistry and Immunohistochemistry

Cells were fixed in 4% paraformaldehyde for 10 min at room temperature, followed by rinsing 3 times with phosphate-buffered saline (PBS). Cells were then permeabilized with 0.1% Triton X-100 in 1XPBS for 10 min at room temperature. After again rinsing 3 times with PBS, cells were incubated with blocking buffer containing 10% goat serum and 10 mg/ml BSA at room temperature for 1 hr. All primary antibodies were diluted in PBS. Cells were incubated with primary antibodies as listed at 4°C for 12-16 hours, followed by the corresponding secondary antibody solutions in 37 °C for 1 hr. Cells were rinsed with 1XPBS three times followed by mounting of coverslips with anti-fade solution (Invitrogen). Primary antibodies used were (dilutions listed in parentheses): mouse anti-Tuj1 (Covance, 1:1000); rabbit anti-Tuj1(Covance, 1:2000); rabbit anti-MAP2 (Sigma,1:400); mouse anti-MAP2 (Sigma, 1:500); mouse anti-Tau (Tau1, Millipore, 1:500); mouse anti-NeuN (Millipore, 1:200); rabbit anti-vGLUT1 (Synaptic System, 1:100); rabbit anti-GAD65 (Millipore, 1:500); chicken anti-Tbr1 (Millipore, 1:500); mouse anti-human neurofilament (Sigma, 1:500); rabbit anti-Pax6 (Millipore, 1:500); mouse anti-Nestin (Millipore, 1:500); mouse anti-Ascl1 (BD Pharmingen, 1:10). Also used were: mouse anti-APP (22C11, Millipore, 1:500), rabbit anti-APP (KDI, Millipore, 1:500), rabbit anti-BACE1 (Covance, 1: 500; this was further purified by protein G sepharose chromatography kit from GE healthcare), mouse anti-BACE1 (3D5, gifts from Robert Vassar), rabbit anti-EEA1 (Millipore, 1:500), mouse anti-M6PR (Abcam, 1:500), rabbit anti-LAMP2 (Sigma, 1:400). Dylight 488-, Dylight 549- and Dylight 649-conjugated secondary antibodies were purchased from Jackson Immunoresearch. Alexa-488, Alexa-633-conjugated secondary antibodies were obtained from Invitrogen. For immunohistochemical analysis of acutely prepared brain sections, the following primary antibodies were used: rabbit anti-GFP antibody (Invitrogen, 1:200), mouse anti-human NCAM (Santa Cruz Biotechnology, 1:50), mouse anti-human mitochondria (hMito, MTC02; Abcam, 1:200).

Imaging was performed by laser-scanning confocal microscopy with a 63x /1.4 objective (LSM510, Carl Zeiss) or by epifluorescence microscope (Olympus 1X71; Japan). hiN cell counts and fluorescence intensities were quantified by taking 10 to 35 images of randomly selected views per well. Subsequently, images were analyzed for cell counts and fluorescent intensity using Image J 1.42q software (National Institute of Health, USA). Values are presented as mean ± SEM.

FACS sorting and RNA extraction

hiN cell cultures (10^6 cells) were detached using TrypLE Express (Invitrogen, CA). After gentle trituration, cells were filtered through cell strainer caps (40µm mesh) to obtain a single cell suspension. Cell were then pelleted by centrifugation at 1000 rpm and resuspended in 50µl staining buffer (PBS, 0.5% bovine serum albumin [BSA], 2mM EDTA and 20mM Glucose). 50µl antibody solution was prepared at 2X concentration (2µl mouse anti human NCAM antibody labeled with a V450 fluorophore [BD Bioscience, CA, 1:50] in 50µl staining buffer). The antibody solution was mixed with the cell suspension in a 1.5ml eppendorf tube and incubated for 15 min at room temperature. The stained cells were washed twice with 1ml staining buffer and again pelleted by centrifugation at 1000 rpm. The pellet was resuspended in 400 µl staining buffer in a FACS tube (BD Bioscience, CA) and placed on ice. Sorting threshold was set up based on control groups (details were described in the legend of Figure S2). Cells were analyzed using a FACS Aria IIu (BD Bioscience, CA). Gating was based on fluorescence intensity of the NCAM-V450 chromophore (at 450 nm) as well as autofluorescence (at 660 nm). FACS of hiN cell cultures in the absence of the NCAM-V450 antibody (negative control; see S2I) defined the unstained population threshold parameters. Subsequently, NCAM-V450 antibody stained hiN cells were gated based on these parameters. Cells were sorted directly into RNA lysis solution (Ambion, TX) by BD FACS Aria IIu (BD Bioscience, CA).

Transcriptome analysis

RNA was extracted from cell preparations using the RNAqueous Micro Kit (Ambion). Concentration and quality of RNA were assessed using the Bioanalyzer (Agilent). mRNA was amplified and labeled using Ovation Pico WTA System (Nugen), and subsequently hybridized to Human Genome U133 Plus 2.0 Arrays (Affymetrix). Raw data were processed using the R statistical computing environment Affymetrix Linear Modeling Graphical User Interface package (affylmGUI; (Smyth, 2004). Normalization was performed using the R AffyPLM module from Bioconductor (www.bioconductor.org) and differential expression using the Bioconductor B-statistic module (to determine the log of odds that a gene is differentially expressed, adjusted for multiple comparisons; (Smyth, 2004). The R hclust package was used for hierarchical clustering of the sample profiles by complete linkage based on the Pearson's correlation coefficient metric. Gene ontology category enrichment was assessed using DAVID online tools (http://david.abcc.ncifcrf.gov/ (Huang et al., 2009).

We also performed hierarchical clustering of the hiN cell sample gene expression profiles with a large set of 336 existing gene expression profiles of human neurons and other cell types that are publically available on the NIH Gene Expression Omnibus Dataset repository (GEO; Figure S3 and Table S4). Individual gene expression profiles (as CEL files) for each of the 336 profiles were downloaded from the GEO website (http://www.ncbi.nlm.nih.gov/geo/) and normalized together with experimental gene expression profiles (as CEL files; microarray experiments as presented in Fig. 2) in a single batch within the GenePattern ExpressionFileCreator function (http://genepattern.broadinstitute.org) using the GC-content Robust Multi-array Average (GCRMA) algorithm. Binary present/absent calls for each transcript probe set within each sample were computed in the same process. 710 Affymetrix probesets that were either present in all FACS-sorted hiN cell samples and absent in all fibroblast samples (as per Figure 2D), or absent in all FACS-sorted hiN cell samples and present in all fibroblast samples, were utilized in the subsequent binary hierarchical clustering analysis. Such

present/absent binary analysis of gene expression constitutes a stringent test for differentially expressed transcripts, and minimizes potential experimental method bias that exists across the varied methods included in the generation of the 336 existing data sets within the GEO repository (see Table S4). Hierarchical clustering was thus implemented using simple binary present/absent calls (Yang et al., 2011) for each of the 710 probe set values within each sample. Binary hierarchical clustering using Pearson's correlation coefficient and complete linkage, as well as subsequent generation of graphics for visualization, were performed using the TM4 MeV function (Saeed et al., 2003).

In utero transplantation

In order to mark transplanted hiN cells, human skin fibroblasts (STC0022) were transduced with a GFP-encoding lentiviral vector 10 days prior to hiN cell induction. After three passages to remove contaminating virus, the fibroblasts were transduced with lentiviral vectors encoding Ascl1, Brn2, Myt1l, Oligo2 and Zic1 as described above. 7 to 10 days after hiN cells induction, hiN cells were trypsinized and triturated to single-cell suspensions in the presence of 0.1% DNase (Qiagen). Timed-pregnant C57BL/6N mice at day 13.5 of gestation were anesthetized with oxygen containing 2% isoflurane administered through an inhalation mask, and 2-5×10^5 cells were injected into the telencephalic vesicle of each embryo as described (Brustle et al., 1997; Wernig et al., 2008).

Transplanted mice were spontaneously delivered and analyzed 1 to 2 weeks after surgery as indicated. Following deep isoflurane anesthesia, mice were decapitated, and the brains were rapidly removed and put in 4% paraformaldehyde for two days for fixation. For immunohistochemistry, 50 µm sections were cut with a vibrating blade microtome.

Electrophysiology and Calcium Imaging

Recordings in cultured cells were performed from hiN cells at 3-4 weeks after viral infection. Tight-seal whole cell recordings (WCR) were performed with borosilicate glass pipettes (resistance 5-8 MΩ). Recordings were made with an Axopatch 200B amplifier (Axon Instruments), and signals were sampled and filtered at 10 Khz and 5 Khz, respectively; whole cell capacitance was cancelled and series resistance compensated 60-80% using standard techniques. The extracellular solution contained: 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES and 10 mM glucose, pH 7.4 adjusted with NaOH. To study Na^+ currents, the intracellular solution used was 135 mM $CsMeSO_4$, 4.1 mM $MgCl_2$, 10 mM HEPES, 10 mM EGTA, 0.4 mM Na-GTP, 3.6 mM Na-ATP (pH 7.4 adjusted with CsOH). To study barium currents the bath solution contained: 132 mM tetraethylammonium (TEA)-Cl, 10 mM $BaCl_2$, 10 mM HEPES, and 10mM glucose (pH 7.4 adjusted with CsOH). To elicit K^+ currents, to view spontaneous voltage clamp events, and in the context of glutamate puff and current clamp recordings, the pipette solution had K^+ replacing Cs^+ as the main cation. GABA puff experiments were performed with a lower Cl^- intracellular solution, approximating physiological levels, as follows: 150 mM Cs-gluconate, 4.6 mM $MgCl_2$, 0.1 mM $CaCl_2$, 1 mM EGTA, 10 mM HEPES-Cs, 0.4 mM Na-GTP, and 4 mM Na-ATP. GABA responses were also elicited using a solution with a Cl^- concentration close to the extracellular solution (data not shown). Its composition was: 150 mM CsCl, 4.6 mM $MgCl_2$, 0.1 mM $CaCl_2$, 1 mM EGTA, 10 mM HEPES-Cs, 0.4 mM Na-GTP, and 4 mM Na-ATP, pH 7.4. Liquid junction potentials were measured and subtracted for generation of current density-voltage plots and to measure passive membrane properties. Voltage dependent currents were recorded both with and without a P/4 protocol (Bezanilla and Armstrong, 1977). Recordings from transplanted cells were performed in acutely prepared horizontal and vertical brain slices through the entire cerebrum (180 µm thick) as described in detail (Llano and Bezanilla, 1980). For glial co-culture studies: murine astroglial cells were obtained from mice ubiquitously expressing red fluorescent protein (Muzumdar et al., 2007). Glial cells were prepared as previously described (Kaech and Banker, 2006) and added into hiN

cultures prepared as above, 2 to 2.5 weeks after viral cocktail transduction. 20,000-25,000 glial cells added/well of a 24-well plate. Recordings from co-cultures were performed on cells with a neuronal morphology that lack red fluorescence, 1-2 weeks after initiation of co-culturing.

For recordings from acutely prepared brain slices after in utero transplantation: animals were sacrificed at postnatal days as indicated. Brain slices were prepared using standard techniques. Recordings were performed at 20-23 ºC in GFP-expressing cells identified by fluorescence microscopy. In the recording chamber, slices were perfused (1.5 ml/min) with a saline solution containing 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM $MgCl_2$ and 10 mM glucose equilibrated with a 95% O_2-5% CO_2 mixture. The pipette solution was the same as that used to elicit K^+ currents above, with the addition of Alexa-598 (Invitrogen) as per the manufacturer's instructions, to allow for visualization of the recorded cells.

Evoked calcium transients

Calcium imaging analysis was performed on cultured hiN cells at 3-4 weeks after viral transduction. Whole cell recordings were performed with a pipette filled with a solution containing 140 mM K-gluconate, 5.4 mM KCl, 4.1 mM $MgCl_2$, 9.9 mM HEPES-K, 0.36 mM Na-GTP, and 3.6 mM Na-ATP. The free Mg^{2+} concentration in this solution was 660 µM considering a KD of 100 µM for the binding of ATP to Mg^{2+} (Baylor and Hollingworth, 1998). Oregon Green–BAPTA 1 (OG1; Molecular probes) was added at a concentration of 100 µM. Fluorescent imaging was done in a digital imaging EM-CCD camera (Andor ixon) and LEDs with specific wavelengths as light source (Cairn). Values are expressed as the percentage of change in fluorescence signal with respect to control, $\Delta F/F0=100X(F-F0)/F0-B)$ where F is the fluorescence at any given time, F0 is the average at the pre-stimulus period, and B is the average value at each time point, of the background fluorescence, from four regions of the imaged field that do not contain any part of the dye-filled cell.

Sandwich ELISAs

APP ELISA was performed using a human APP ELISA kit (Invitrogen, Camarillo, CA), according to the manufacturer's instruction. Absorbance was read on a VersaMax ELISA Microplate Reader (Molecular Devices, Inc. Sunnyvale, CA) at 450 nm. The amount of APP was normalized to the total cell protein (determined with the DC Protein Assay Reagent kit; Bio-Rad, Hercules, CA). sAPPβ and Aβ ELISA were performed on supernatant media from hiN cell cultures at 21 days after viral transduction using BetaMark™ sAPP Beta ELISA kit (Covance, Princeton, NJ), according to the manufacturer's instruction. The chemiluminescence was read on a microplate luminometer (SPECTRAFluoR Plus, TECAN, Männedorf Switzerland). Aβ quantification was performed by ELISA as described previously (Cirrito et al., 2003). Media was conditioned for 48 hr prior to harvesting. Samples were analyzed for Aβ40 or Aβ42 using specific sandwich ELISAs. Briefly, Aβ40, and Aβ42 were captured using monoclonal antibodies targeted against amino acids 35-40 (HJ2.0), or 33-42 (HJ7.4) of Aβ, respectively. The antibodies HJ2.0, HJ5.1 and HJ7.4 were gifts from David M. Holtzman. For Aβ40 and Aβ42 assays, a biotinylated central domain monoclonal antibody (HJ5.1) followed by streptavidin-poly-HRP-40 was used for detection (Sigma). All assays were developed using Super Slow ELISA TMB (Sigma) and read on a VersaMax ELISA Microplate Reader (Molecular Devices, Inc. Sunnyvale, CA) at 650 nm. ELISA signals were reported as the mean ± SEM of three replica wells in ng of Aβ per ml supernatant, based on standard curves using synthetic Aβ40 and Aβ42 peptides (rPeptide; Bogart, GA) Samples was optimized to detect Aβ40 and Aβ42 in the ranges of 1-3,000 ng/ml and 0.03–30 ng/ml, respectively (Figures S5F and S5G). The amount of sAPPβ and Aβ was normalized to the cell number per well as indicated.

For Dot blot analysis to confirm antibody specificity, membranes were blocked in 10% skimmed milk in TBST (10 mM Tris, 150 mM NaCl, 0.1% Tween 20, pH 7.4) for 1 hour at room

temperature, then incubated with a 1 µg/ml dilution of each Aβ specific antibodies (HJ2.0, HJ5.1, HJ7.4) and 1:500 dilution of mouse monoclonal Aβ antibody (4G8, Covance, Emeryville, CA) in 10% skimmed milk in TBST at 4°C overnight. The following day, the membranes were washed in TBST and then incubated with HRP-conjugated Donkey anti-mouse IgG (Jackson ImmunoResearch laboratory Inc., West Grove, PA) at a dilution of 1:2500 for 1hr at room temperature. The membranes were then washed in TBST and immunoreactive protein signals were visualized by enhanced chemiluminescence (ECL) reagents (Pierce Biotechnology, Rockford, IL).

Quantitative real time RT-PCR

Quantitative real time RT-PCR was performed as described (Rhinn et al., 2008). Gene expression levels were quantified by the ΔΔCt method (Rhinn et al., 2008) using primers for OTX2 (Fwd: GAC CCG GTA CCC AGA CAT C, Rev: TGG CCA CTT GTT CCA CTC TC), FOXG1 (Fwd: AGA AGA ACG GCA AGT ACG AGA, Rev: TGT TGA GGG ACA GAT TGT GGC), GAPDH (Fwd: GGT CTC CTC TGA CTT CAA CA, Rev: GTG AGG GTC TCT CTC TTC CT), SYP (Fwd: AGG GAA CAC ATG CAA GGA G , Rev: CCT TAA ACA CGA ACC ACA GG)), BACE1 (Fwd: CAG TCC TTC CGC ATC ACC, Rev: TGA CAG CAA AGC CAA TTC GT), and APP (Fwd: AAC CAG TGA CCA TCC AGA AC, Rev: ACT TGT CAG GAA CGA GAA GG). For detection of viral transduction factors, forward primers used were:
Ascl1 (Fwd: CGG TGA GCG CTG CCT TTC A), Brn2 (Fwd: CAG GAG ACA GAA AGA GAA AAG GAT GAC), Myt1l (Fwd: GAT GGG TCA GGA CAC GTC AGT), Zic1 (Fwd:CTG CAC ATC ACG GGG CTG GA). For selective detection of expression of extrinsic viral-encoded factors, a common reverse PCR primer was used: GTA GAA TCG AGA CCG AGG AGA G.

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Supplemental Table S2

Probeset	Gene Symbol	Fibro_0022	Fibro_7768	iN_Mix_0022	iN_Mix_7768
200953_s_at	CCND2	4.86	4.87	9.94	9.59
202668_at	EFNB2	2.82	2.47	6.77	7.43
202672_s_at	ATF3	2.60	3.09	3.56	3.35
208131_s_at	PTGIS	6.49	6.40	3.34	3.34
211356_x_at	LEPR	5.12	5.50	2.93	2.77
212022_s_at	MKI67	5.83	5.70	2.60	2.59
228153_at	RNF144B	2.77	2.60	4.69	4.56
233822_x_at	NA	9.13	9.63	9.87	9.89
205249_at	EGR2	2.46	2.46	2.46	3.01
212670_at	ELN	2.67	2.44	6.80	6.99
203394_s_at	HES1	2.99	2.84	2.76	3.72
203395_s_at	HES1	3.12	3.03	4.22	4.39
205828_at	MMP3	11.01	12.73	4.06	3.92
204105_s_at	NRCAM	2.92	2.40	5.92	5.93
204159_at	CDKN2C	4.66	4.82	2.48	2.48
229490_s_at	NA	6.28	5.80	2.95	2.73
206172_at	IL13RA2	7.91	6.71	2.66	2.66
223315_at	NTN4	2.89	2.87	9.01	7.66
222608_s_at	ANLN	6.30	6.89	3.30	3.30
200952_s_at	CCND2	2.45	2.45	6.43	5.72
1561775_at	NA	6.37	7.35	6.46	7.28
227760_at	IGFBPL1	2.46	2.57	8.98	7.80
1554828_at	PDGFRA	5.03	5.12	2.83	2.93
204631_at	MYH2	3.08	3.08	7.46	7.20
205203_at	PLD1	4.85	4.72	2.59	2.67
205352_at	SERPINI1	2.46	2.40	7.09	7.64
209099_x_at	JAG1	2.45	2.65	6.08	7.44
209101_at	CTGF	7.68	7.69	11.23	11.71
211959_at	IGFBP5	7.48	6.78	9.80	9.75
212158_at	SDC2	5.15	5.56	8.33	8.77
218039_at	NUSAP1	6.34	7.53	3.17	2.85
229538_s_at	IQGAP3	4.88	4.11	2.40	2.40
227140_at	NA	4.81	5.34	8.42	8.93
209032_s_at	CADM1	4.44	4.27	8.19	8.32
209613_s_at	ADH1B	4.78	4.66	2.42	2.47
210833_at	PTGER3	3.43	3.51	5.68	5.79
216268_s_at	JAG1	3.22	2.57	6.25	7.68
218755_at	KIF20A	5.53	5.88	2.46	2.46
222725_s_at	PALMD	2.47	2.41	6.17	5.88
228575_at	IL20RB	2.85	2.82	3.99	3.74
202503_s_at	KIAA0101	5.15	4.86	2.42	2.48
211080_s_at	NEK2	5.36	4.94	2.40	2.42
217183_at	LDLR	5.85	6.07	4.42	4.42
242425_at	NA	4.38	4.20	3.24	3.35
207414_s_at	PCSK6	2.58	2.49	4.10	3.62

Supplemental Table S4

Label
EntCtx_LayerII_Ctl_1
EntCtx_LayerII_Tangle_1
EntCtx_LayerII_Ctl_2
EntCtx_LayerII_Tangle_2
EntCtx_LayerII_Ctl_3
EntCtx_LayerII_Tangle_3
EntCtx_LayerII_Ctl_4
EntCtx_LayerII_Tangle_4
EntCtx_LayerII_Ctl_5
EntCtx_LayerII_Tangle_5
EntCtx_LayerII_Ctl_6
EntCtx_LayerII_Tangle_6
EntCtx_LayerII_Ctl_7
EntCtx_LayerII_Tangle_7
EntCtx_LayerII_Ctl_8
EntCtx_LayerII_Tangle_8
EntCtx_LayerII_Ctl_9
EntCtx_LayerII_Tangle_9
EntCtx_LayerII_Ctl_10
EntCtx_LayerII_Tangle_10
EntCtx_layerIII_1
EntCtx_layerIII_2
EntCtx_layerIII_3
EntCtx_layerIII_4
EntCtx_layerIII_5
EntCtx_layerIII_6
EntCtx_layerIII_7
EntCtx_layerIII_8
EntCtx_layerIII_9
EntCtx_layerIII_10
EntCtx_layerIII_11
EntCtx_layerIII_12
EntCtx_layerIII_13
Hipp_layerIII_1
Hipp_layerIII_2
Hipp_layerIII_3
Hipp_layerIII_4
Hipp_layerIII_5
Hipp_layerIII_6
Hipp_layerIII_7
Hipp_layerIII_8
Hipp_layerIII_9
Hipp_layerIII_10
Hipp_layerIII_11
Hipp_layerIII_12